increased on the same diet as that in the normal dogs, and the cholesterin is decreased.

That the formation of urobilin in the fæces is diminished in the absence of the large intestine; the sulphates vary the same as the normal as regards those combined with the alkalis, while those combined with the aromatic substances are markedly diminished, showing that intestinal putrefaction is decreased.

"Further Observations concerning the Relation of the Toxin and Anti-Toxin of Snake-Venom." By CHARLES J. MARTIN, M.B., D.Sc., Acting Professor of Physiology in the University of Melbourne. Communicated by W. D. HALLIBURTON, F.R.S. Received August 23, 1898, and published during the Vacation.

The discrepancy between the quantities of anti-venene required to neutralise a given dose of venom when they are (1) previously mixed outside the body, and (2) simultaneously injected under the skin in different parts of the body, has been drawn attention to by Fraser and myself. My experience coincides with Fraser's* upon this point, viz., that it requires at least 10—20 times as much anti-venene to counteract a given dose of venom when they are injected separately, but at the same time, as is necessary to effect this if they are mixed together prior to injection.

Sometimes, however, the quantity necessary by simultaneous but separate injection may be much greater; in one of Fraser's experiments 1000 times as great.† Moreover, there is no constant ratio between the amounts necessary under the two conditions, as will be seen from the experiments tabulated below (Series A). In this series, experiments 1—4, in which increasing doses of venom were employed, show that 0·5 c.c. of the particular sample of serum used was more than adequate to prevent a fatal result when previously mixed for fifteen minutes at temperature 13° C. with 0·5 c.c. of a solution containing 0·0001 gram of the venom per c.c. As 0·00003 gram per kilo was found to be the minimal fatal dose of this poison, one may be sure that under these conditions 0·5 c.c. of the serum is adequate to neutralise more than 0·00002 gram of the venom, that is, 0·00005 gram *minus* one fatal dose.

In experiments 5—12, 0·00005 gram of venom per kilo. was injected in each case, and increasing amounts of serum separately, but at the same time. Under these conditions, every quantity less than 8 c.c., that is, sixteen times as much as is fully adequate to prevent any symptoms when brought directly into contact with the poison before

* 'Nature,' April 23, 1896.

† *Loc. cit.*, p. 594.

injecting, failed to counteract the venom. Even as much as 9 c.c. and 15 c.c. was equally useless, although the animals in the experiments in which 8 c.c. and 10 c.c. were employed lived.

Series A.

	Amount of venom per kilo. of rabbit.	Amount of serum per kilo.		Result.
1	0·00005 gram	0·5 c.c.	Mixed together for 15 minutes prior to injection.	Lived.
2	0·00006 ,,	0·5 ,,		Lived.
3	0·00007 ,,	0·5 ,,		Died.
4	0·00008 ,,	0·5 ,,		Died.
5	0·00005 ,,	5·0 ,,	Injected simultaneously but separately.	Died.
6	0·00005 ,,	6·0 ,,		Died.
7	0·00005 ,,	7·0 ,,		Died.
8	0·00005 ,,	8·0 ,,		Lived; very ill for 3 days.
9	0·00005 ,,	9·0 ,,		Died.
10	0·00005 ,,	10·0 ,,		Lived.
11	0·00005 ,,	15·0 ,,		Died.
12	0·00005 ,,	20·0 ,,		Lived.

The further experiments detailed in the present paper afford a reasonable interpretation of the very different efficacy of anti-toxic serum under these two conditions. They are also an additional confirmation of the conclusions regarding the direct chemical nature of the antagonism between the toxins and anti-toxins of diphtheria and snake-poison respectively, which were drawn by Cherry and myself in a recent paper.* Moreover, some inferences which seem to me to be necessitated by the experimental results are of practical importance in the treatment of snake-poisoning, and not devoid of interest in their bearing upon the relations of toxins and anti-toxins in general.

The experiments arranged in tabular form below were made with the object of obtaining definite data concerning the proportions of anti-toxin to toxin necessary to save an animal under the following three conditions:—

* 'Roy. Soc. Proc.,' vol. 63, p. 420, 1893.

(1) Mixed together prior to injection.
(2) Injected simultaneously, the anti-toxin into a vein, and the venom subcutaneously.
(3) Injected simultaneously, but separately, under the skin.

The venom of *Hoplocephalus curtus*, the Australian tiger-snake, was used. After weighing the dried venom, it was dissolved in enough 0·9 per cent. NaCl solution for 1 c.c. to contain 0·0001 gram of the venom. This solution was heated momentarily to 90° C. in order to destroy one of the poisonous constituents which coagulates at 85° C.* The poisonous proteose remaining produces the same symptoms as cobra poison, and is very probably identical with the principal poisonous constituent in that venom.

The anti-venene was prepared by Dr. Calmette. Two quite different serums were used. For the experiments in Series A above, samples dated November, 1896, were employed, and for the experiments in Series B samples bearing date December, 1897. The anti-toxic value of the former, according to Behring's method of notation, I found to be 1/50th of a normal unit per c.c.; of the latter 1/200th of a normal unit per c.c.†

The control experiments are in Table I. Here the same proportions of venom to body weight, as employed in the experiments in Tables II, III, and IV, were injected, but no serum given. The effect of these doses of venom upon the rectal temperature and the time they took to kill is shown for comparison. From these experiments it is seen that 0·00003 gram venom per kilo. is just on the margin of fatality. From other experience I have found that this quantity generally kills.

II and III show parallel series of experiments. The amount of anti-venene per kilo. remains constant, but the quantities of venom in each case increase from 0·00003 to 0·00008 gram per kilo. In II the venom and anti-venomous serum were mixed together in a glass and allowed to remain in contact for fifteen minutes at temperature 13° C. prior to injection. In III the anti-venene was injected into the jugular vein at the same time that the venom solution was placed under the skin. II and III are similar in every other respect. The solution of venom and the serum used were the same, and the experiments were made at the same time.

The two experiments in IV, made with the same venom solution and serum, show for comparison the result of injecting the venom and anti-

* C. J. M., 'Roy. Soc. N.S.W. Proc.,' August, 1896.

† For the latter sample I am indebted to the kindness of Dr. Calmette. The anti-toxic value mentioned above refers to the serum as it arrives in Australia, and titrated against the venom of *Hoplocephalus curtus*, heated to 90° C. I believe, from Dr. Calmette's statement, that the serum must be much more effective against cobra poison, or else that it deteriorates before reaching me.

Series B. Table I.—Control Experiments with Venom Solution only.

	Weight of rabbit in grams.	Amount of venom per kilo.	Amount of serum per kilo.	Temp. at time of injection.	Result.						
					Temp. 12 hours after.	Temp. 24 hours after.	Temp. 36 hours after.	Temp. 48 hours after.	Temp. 60 hours after.	Temp. 72 hours after.	
		gram.		°	°	°	°	°	°	°	
1	1300	0·00003	..	39·5	39·2	38·3	38·3	36·5	..	..	Died in 50—54 hours.
2	1230	0·00003	..	39·7	39·7	38·7	38·7	38·8	39	39·7	Lived.
3	1120	0·00005	..	39·8	38	..	..	..	..	..	Died 26 hours.
4	1190	0·00006	..	39·2	..	..	..	..	..	..	Died 13 hours.
5	1150	0·00008	..	40·1	..	..	..	..	..	..	Died 11 hours.

Table II.—In which the Venom Solution and Anti-venene were mixed together for 15 Minutes prior to Injection under the Skin.

		gram.	c.c.	°	°	°	°	°	°	°	
6	1230	0·00003	2	39·8	39·6	39	39·8	39·8	..	..	Lived.
7	1300	0·00004	2	39·8	39·7	39·6	39·8	39·9	39·8	..	Lived.
8	1330	0·00005	2	40	39·8	39·6	38·2	35	..	..	Died 53 hours.
9	1108	0·00006	2	40·2	39·4	38·4	39·8	39·4	38·5	34	Died 75 hours.
10	1112	0·00007	2	39·5	37·5	36·0	..	..	..	..	Died 27 hours.
11	1190	0·00008	2	39·25	37·6	36·4	..	..	..	..	Died 27½ hours.

Table III.—In which the Anti-venene was injected into the Jugular Vein at the same time that the Venom Solution was introduced under the Skin.

	Weight of rabbit in grams.	Amount of venom per kilo.	Amount of serum per kilo.	Temp. at time of injection.	Result.						
					Temp. 12 hours after.	Temp. 24 hours after.	Temp. 36 hours after.	Temp. 48 hours after.	Temp. 60 hours after.	Temp. 72 hours after.	
		gram.	c.c.	°	°	°	°	°	°	°	
12	1230	0·00003	2	39·5	39·5	39·6	39·6	39·7	..	39·6	Lived.
13	1260	0·00004	2	39·8	39·6	39·¼	39·9	40	..	39·9	Lived.
14	1260	0·00005	2	39·5	39·5	38·5	39·5	39·5	..	39·6	Lived.
15	1240	0·00006	2	39·8	37·2	35·0	..	..	..	..	Died 26 hours.
16	1300	0·00007	2	39	36·8	30	..	..	..	..	Died 24¼ hours.
17	1310	0·00008	2	40	33	..	..	..	..	..	Died 20 hours.

Table IV.—In which the Venom and Anti-venene were injected separately but simultaneously under the Skin.

		gram.	c.c.	°	°	°	°	°	°	°	
18	1100	0·00005	10	40	36·2	..	..	..	..	..	Died 15 hours.
19	1140	0·00005	20	39·7	36·8	..	..	..	..	..	Died 19 hours.

venene simultaneously, but separately, under the skin on different sides of the body. These large quantities were introduced by injecting about 2 c.c. into different situations; 20 c.c. of serum is quite harmless.

The rectal temperature of each rabbit was taken at the time of injection and each twelve hours subsequently. The fall in temperature caused by the poison is a good indication of the extent to which the animal is affected.

The conclusions I feel justified in drawing from the above experiments are:—

(1) That about the same quantity of anti-venene necessary to neutralise the venom *in vitro*, is capable of doing so when the former is injected into the blood-stream, and the latter subcutaneously.

(2) At least ten to twenty times this quantity is required when they are both placed simultaneously under the skin, but in different parts of the body.

That the proportion of toxin to anti-toxin necessary to neutralise the former should be approximately the same whether they be (1) mixed in a glass, or (2) the anti-toxin be injected into the blood-stream and the toxin subcutaneously, might be expected if the nature of the antagonism between them be a chemical one, and in consideration of the evidence adduced by Kanthack,* Erhlich,† Fraser,‡ Stevens, and Meyer,§ and Cherry and myself,‖ I do not see that one can come to any other conclusion.

The toxin and anti-toxin of snake-poison neutralise one another when mixed together in adequate proportions, quite irrespective of the actual quantity of each. Solutions of the two can be titrated against each other just like standard solutions with the life of a rabbit as an indicator, in which the error in the determination of the "end-point" is one fatal dose.

If anti-venene be introduced into the blood-stream the anti-toxin is there ready to neutralise the toxin as it is absorbed, and, as might have been predicted, the amount found necessary by titration outside the body is just about adequate to neutralise the toxin as it makes its appearance in the blood. The experiments indicate, however, that a slightly larger proportion of anti-toxin is necessary under these circumstances, for the rabbits 9, 10, 11 lived a little longer than rabbits 15, 16, 17. This result may very well be due to delayed chemical action due to the dilution of the anti-toxin in the blood.

* Quoted by Stevens and Meyer, 'Path. Soc. Lond. Proc.,' March 1, 1898.
† 'Fortschr. der Med.,' 1897, No. 2.
‡ *Loc. cit.*
§ 'Path. Soc. Proc.,' March 1, 1898.
‖ *Loc. cit.*

The much higher proportion of anti-toxin to toxin required when separately introduced under the skin seems to necessitate the inference that anti-toxin is comparatively slowly absorbed from the subcutaneous spaces. Our chemical knowledge of this poison in *Hoplocephalus* venom and of the active principle in anti-venene, together with what is known of the physiological mechanism of absorption, is quite in accordance with the view that this anti-toxin is only capable of slowly penetrating the capillary wall, whereas the venom passes through fairly rapidly. The constituent of the venom which was used in the above experiments is an albumose. It dialyses slowly, can be filtered through a film of gelatin under pressure, although it does not pass through so readily as water or bodies of simpler molecular constitution.* It is rapidly absorbed by the blood-vessels. An animal can be killed by subcutaneous injection of a large dose in a few minutes, and the result is not retarded by previous ligature of the lymphatics from the limb and the thoracic duct.†

On the other hand, Brodie‡ filtered anti-toxic serum of diphtheria through gelatin, and found that the active properties of the serum remained with the proteids on the outside of the filter. Cherry and I confirmed this result with diphtheria anti-toxin, and found the same for anti-venene,§ and I think both these anti-toxins are bodies of great molecular size comparable to proteids. The walls of the capillaries of the limbs are membranes possessed of permeabilities approximating to those of a film of gelatin, for Starling showed they were relatively although not absolutely impermeable to proteids.‖ If molecular size is the obstacle to proteid absorption from subcutaneous spaces the same would apply to anti-toxins.

Calmette has made the statement that anti-venene is more rapidly absorbed than venom. He does not adduce any experimental proof for such a statement, and I cannot see that the results detailed in this paper can bear any other interpretation than that the poison with which I have been working is absorbed 10—20 times as rapidly as the active principle in anti-venomous serum.

The practical indication of this in the treatment of snake bite is to inject the serum intravenously, until the potency of the anti-venomous serum which is at the disposal of the public is greatly enhanced.

* C. J. M., 'Roy. Soc. N.S.W. Proc.,' Aug., 1896.
† C. J. M., 'Roy. Soc. N.S.W. Proc.,' July, 1895.
‡ 'Journ. of Path.,' 1897.
§ *Loc. cit.*
‖ 'Journ. of Physiol.,' vol. 19, 1895-96, p. 311.